Isolation, production and validation of the first GMP RSV-B virus for use in Controlled Human Infection Studies

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INTRODUCTION & OBJECTIVES

Respiratory Syncytial Virus (RSV) is a significant cause of respiratory infections, particularly in infants and immunocompromised individuals. To facilitate the development of novel therapeutics and vaccines, human challenge trials with well-characterised challenge agents are essential. **Challenge studies with the A RSV strain, Memphis 37, have played an important role in the development of RSV vaccines as well as the efficacy testing of numerous candidate antiviral drugs**. With both RSV A and B serotypes in circulation and of equal importance as a human pathogens, the availability of both RSV A and B challenge strains is essential to facilitate the full use of challenge studies to test candidate vaccines and antivirals. Typically, one or the other serotype dominates in circulation in any given season making it difficult to demonstrate efficacy against both serotypes in field-based studies. The availability of both serotypes as challenge strains enables challenge studies to be used to complement phase 2/3 field trial efficacy studies. **To this aim, we have developed a new RSV B challenge strain.** Here we detail the isolation, production, and testing of a cGMP-produced RSV B serotype strain, RSV-B London as well as the emerging results of virus in a controlled human infection models (challenge study).

METHODS

Virus Selection and Isolation

In 2022, following parental informed consent, bronchoalveolar lavage (BAL) samples were collected from RSV-positive hospitalised infants and put through a triage process to determine their suitability for challenge virus production. Where possible, fresh samples were collected from infected patients, which facilitated aliquoting of the sample before freezing. Aliquoting enabled sample triage to be conducted on one aliquot while retaining others for seed virus production to keep the number of passages required to produce the Challenge virus batch to a minimum. As shown in Figure 1, the triage process involved testing not only the viability of the virus within the clinical sample but also compared the ability to isolate the virus on GMP Wi-38 cells as well as investigating the viral genome sequence and checking for adventitious agents.

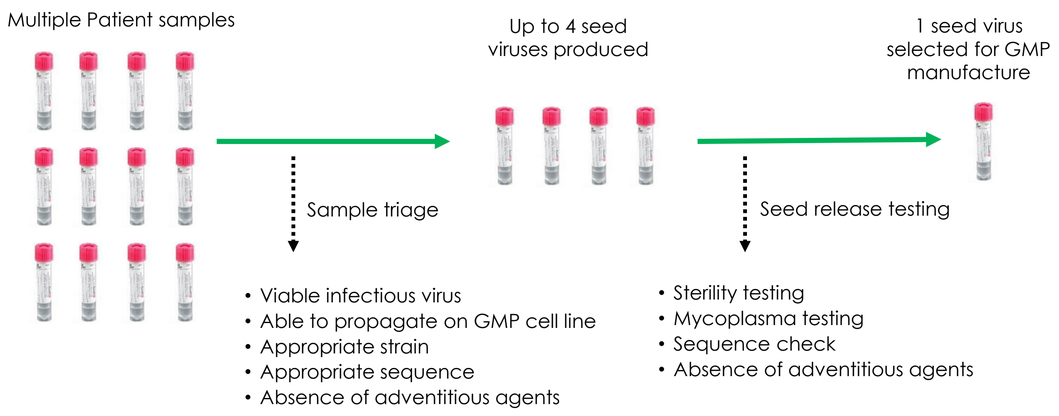

Figure 1: Clinical sample triage process to select appropriate viral source for challenge virus stock production.

Virus was isolated and seed virus stocks produced for selected isolates in an R&D laboratory applying appropriate GMP principles of reagent selection and laboratory conditions to maintain integrity and suitability of the viral stock.

Manufacturing Process

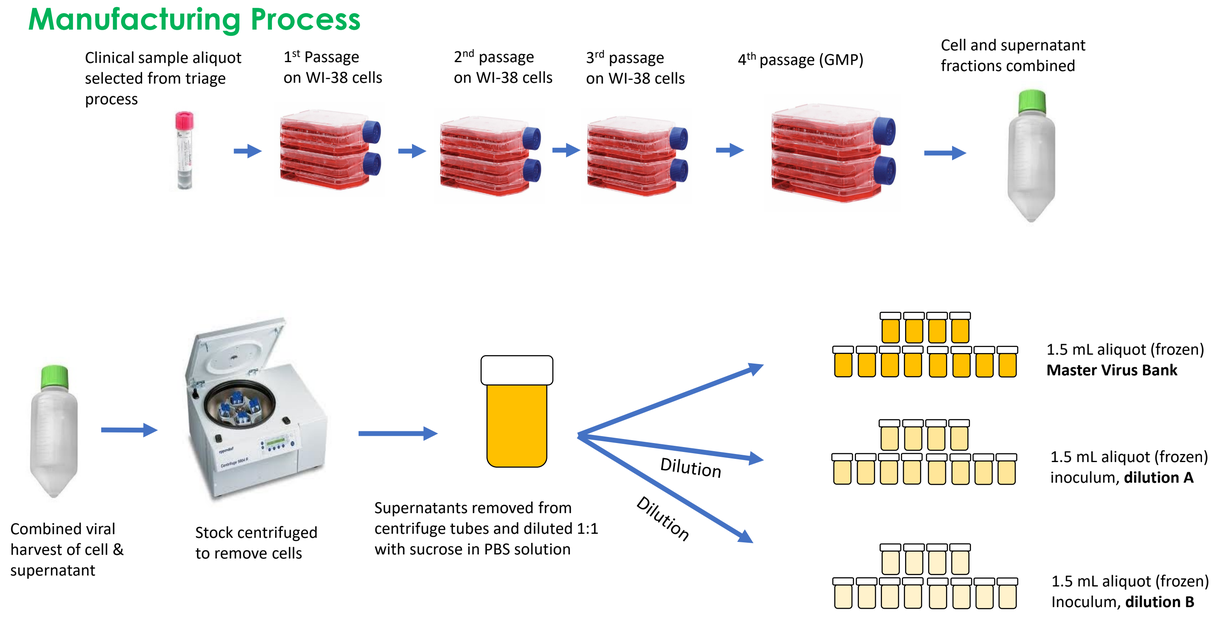

Figure 2: Virus isolation and manufacturing process schematic.

The GMP Challenge virus was produced from a total of 4 rounds of amplification in GMP Wi-38 cells. After the viral-induced CPE had reached >70% the virus was harvested (~ 5 days p.i.). The harvesting procedure included the collection of both the supernatant and cell fractions to collect virus that had both been released from infected cells and that which remained cell-associated. The viral harvest was clarified by centrifugation to remove cellular debris before dilution in a GMP sucrose diluent and subsequent GMP aliquoting of the Master Virus Bank (MVB) and inoculum vials produced by additional dilution of the MVB, all of which were snap frozen before long term storage at -80 °C.

RESULTS

GMP Viral Challenge Stock Release Testing

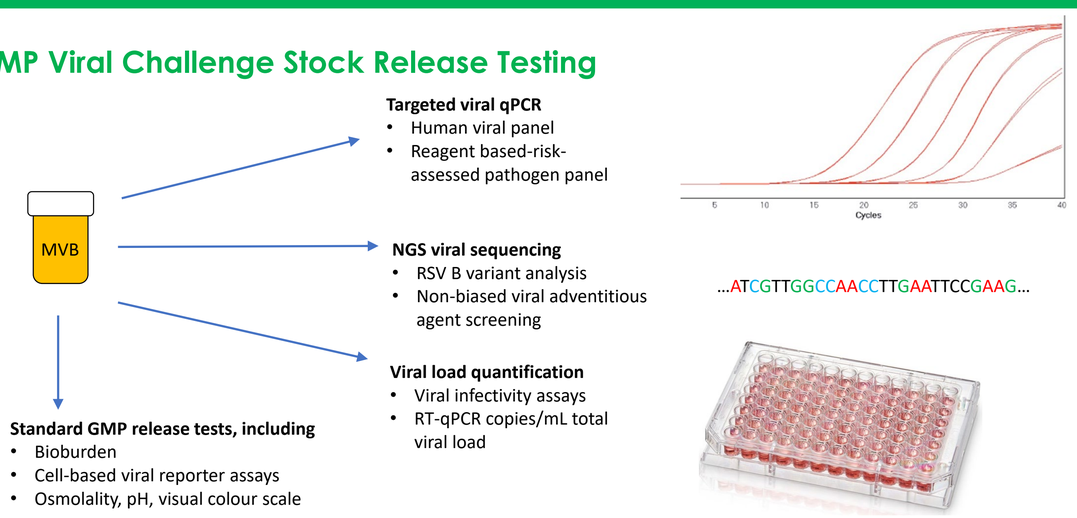

Figure 3: Adventitious agent and release testing strategy summary.

The RSV-B London Challenge virus batch was demonstrated to be safe and suitable for use as a viral challenge agent by a wide range of adventitious agent and release tests (see **Figure 3**). Infectivity of the virus and the ability to propagate in primary respiratory cells was further assessed by inoculation of human airway epithelium cells with the RSV-B London virus. To aid in interpretation of the results, the replication of RSV-B London was compared to RSV-A Memphis 37b, which has already proven safe and efficacious as a challenge virus in CHIM studies. The results demonstrated viral replication in HAE cells were comparable between the two viral strains (**Figure 4 B**).

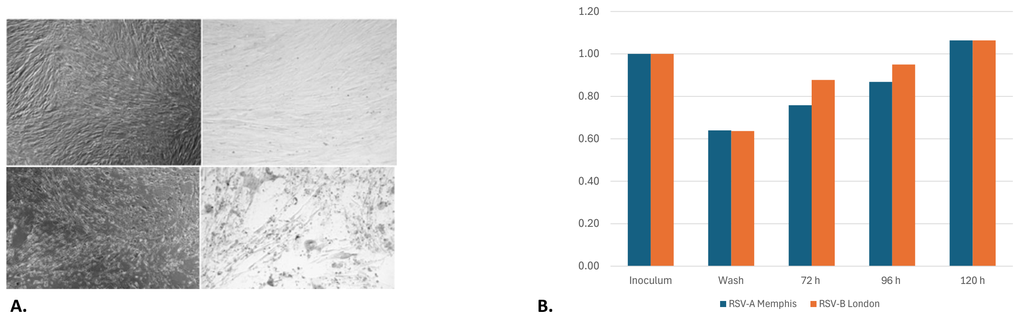

Figure 4: RVS-B London infectivity assessments. **A**. Infection of Wi-38 cells, top row: uninfected cells, bottom row infected cells. **B.** HAE viral load titres as a proportion of the inoculation titre assessed from cell washes collected at 72, 96 and 120 hours post inoculation.

Viral Challenge Model

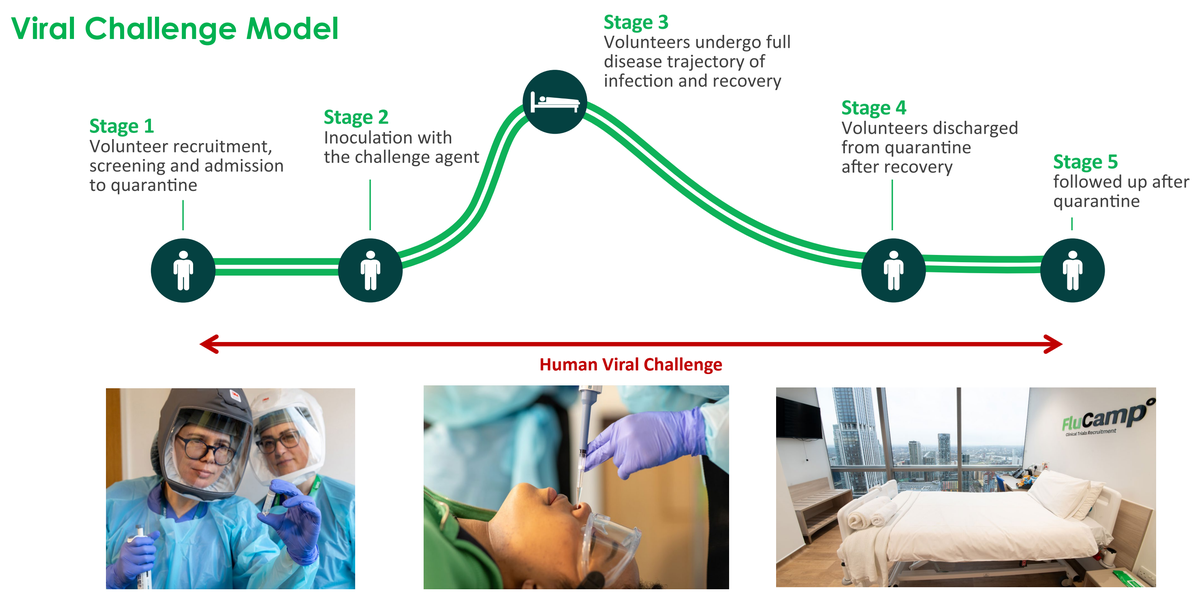

Figure 5: Human challenge model/Controlled human infection model concept.

SCREENING PHASE	INPATIENT PHASE: QUARANTINE/ISOLATION, HUMAN VIRAL CHALLENGE (HVC)				OUTPATIENT PHASE
Day -90 to Day -2/-1 Screening	Day -2/-1 Quarantine admission	Day 0 Inoculation with challenge agent	Days 1 to 12 Assessments	Day 12 Discharge from quarantine unit	Day 28 (±3 days) Follow-up visit

Clinical Outcomes

The viral characterisation study designed to determine the safety and suitability of the RSV-B London virus has recently started with 8 subjects having been inoculated to date and continues.

- Volunteers were monitored closely throughout the infection time course
- No SAEs, excellent safety profile
- No medical concerns
- The virus was well tolerated
- Typical RSV symptoms, similar to those observed with A/Memphis 37

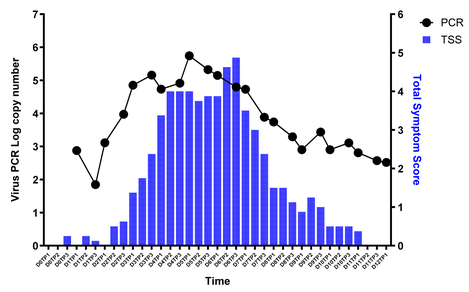

Figure 6: Viral Load (Nasal Wash) and Symptom profile.

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CONCLUSIONS

We have successfully isolated, amplified, and manufactured under cGMP conditions the RSV-B London Challenge Virus, ensuring its safety, infectivity and suitability for use in human challenge trials. Emerging clinical data from the characterisation study indicate **an excellent safety profile with expected viral load and symptomatology.**

To our knowledge this is the **first time an RSV-B has been experimentally administrated to volunteers.** We believe this new virus can complement the successful RSV-A Memphis 37B that has been widely used in helping fast tracking RSV vaccines and antivirals. **The RSV-B London strain is contemporaneous to the currently circulating strains and was produced in human cells so there was no possible host adaptation**.